Instructions for Use

REF - KM0009 ***Helicobacter pylori* Agar**

Dehydrated Culture Media

1. Intended Use

For *in vitro* diagnostic use only. KM0009 *Helicobacter pylori* Agar is a selective medium for the isolation of *Helicobacter pylori* from clinical samples.

2. Composition*

Ingredient	g/L
Peptone	23.0
Sodium chloride	5.0
Charcoal	4.0
Ferrous sulphate	0.25
Sodium pyruvate	0.25
Agar	12.0
Sodium carbonate	0.5

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Helicobacter pylori is found in the digestive tract and can cause stomach ulcers, and in some cases, lead to stomach cancer. *H. pylori* can be contracted from food, water, utensils, or through contact with the saliva or other body fluids of infected people. It is more common in countries or communities that lack clean water or good sewage systems. *Helicobacter pylori* Agar can be used to manufacture a selective plated medium for the isolation of *H. pylori* from clinical samples. The medium is based on a modification of Campylobacter CCDA Blood Free Medium with charcoal, ferrous sulphate and sodium pyruvate replacing the horse blood, and can be made selective for *H. pylori* isolation by the addition of 10% horse serum, cefsulodin, vancomycin and amphotericin [1].

4. Principle

Selectivity of *Helicobacter pylori* Agar is achieved by the addition of vancomycin and cefsulodin, to suppress other bacteria, and amphotericin to inhibit yeasts [1 2]. Horse serum (10%) is also added to promote optimum growth of *Helicobacter* species. Plates should be Incubate in micro-aerophilic conditions at 37 ± 1°C for 48-72 hours.

5. Preparation Instructions

Suspend 45.0 of dehydrated culture medium in 1 litre of deionised/purified water and heat with frequent agitation and boil for one minute to completely dissolve the medium.
Autoclave to sterilise at 121°C for 15 minutes before cooling in a water bath to 45-50°C.
Aseptically add the appropriate volume of *Helicobacter pylori* selective supplement (LS0031) and 10% horse serum.
Gently homogenize the final medium before aseptically dispensing the specified volume into appropriate sterile containers and allowing to cool.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Black fine powder	Black firm gel
pH	N/A	pH = 7.4 ± 0.2

7. Materials Provided

KM0009 can be provided in the formats detailed below. Each tub is labelled with product name, product code, lot number, expiry date, instructions, and appropriate warnings.

Product Code	Product Format
KM0009-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0009-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0009-10KG-10000	1 x 10kg Dehydrated Culture Media Tub

8. Materials Needed but not Provided

Standard microbiological laboratory materials *e.g.,* autoclave, sterile loops or swabs, collection containers, incubators, and quality control organisms.

9. Specimens

KM0009 *Helicobacter pylori* Agar can be used with the following specimens:

Clinical: Gastric biopsies from the stomach or upper intestine collected via endoscopy, or stool samples.

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

Note - the recovery of *H. pylori* from gastric biopsies is improved by direct cultivation as soon as possible after collection.

10. Test Procedures and Interpretation of results

If the *Helicobacter pylori* Agar plates have been refrigerated, allow the plates to come to room temperature before inoculation.

Based on sample type and information provided, check to see if the specimen needs to be pre-enriched prior to inoculation to the prepared medium.

Clinical Specimens:

Inoculate specimen, or pre-enriched sample, directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker. Incubate under micro-aerophilic conditions at 37 ± 1°C for 48-72 hours.

After incubation, examine agar for colonies (typical colony appearance outlined in Quality Control table below). Perform further biochemical or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
H. pylori (NCTC 11916)	Micro-aerophilic conditions at 37 ± 1°C for 48-72 hours	Growth: Grey colonies
E. faecalis (NCTC 12697)	Micro-aerophilic conditions at 37 ± 1°C for 48-72 hours	Inhibited
C. albicans (NCPF 3179)	Micro-aerophilic conditions at 37 ± 1°C for 48-72 hours	Inhibited

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium, and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

12. Performance

To fully verify KM0009 *Helicobacter pylori* Agar performance, dehydrated culture media samples were used to prepare *Helicobacter pylori* poured plates and tested to assess colour and colony morphology and recovery level (where an acceptable range is ≥50% and ≤120%) compared to a non-selective reference medium. Samples were inoculated with 30-150cfu for the target organisms and 10^4-10^5 cfu for the non-target organisms then incubated at 35°C-37°C for 48-72 hours. All samples of prepared media produced showed expected recovery and morphology of the required test organism: *Helicobacter pylori* (NCTC 11916), and no recovery of the non-target test organisms: *Enterococcus faecalis* (NCTC 12697) and *Candida albicans* (NCPF 3179). Therefore, it can be concluded that KM0009 *Helicobacter pylori* Agar, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request

13. Limitations of the Media

- Prepared *Helicobacter pylori* Agar may grow bacteria other than *H. pylori* including other *Helicobacter* species and some commensal microflora. Presumptive identity should be confirmed by biochemical, serological, or molecular means.
- Due to natural variation, some strains may grow poorly on this medium.

14. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSSH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

Refer to KM0009 Material Safety Data Sheet.

15. Storage conditions and Shelf life

Store product in the original container with the lid tightly closed at between 10 and 30°C in low humidity conditions away from direct sunlight. Kept under these conditions, the product may be used up to the date of expiry shown on the product label.

Do not use if the product is not free-flowing or displays any sign of colour change, formation of large lumps or hardening of the powder. Additionally, do not use medium if it has been stored inappropriately, the packaging has been damaged or has passed the expiry date.

Dehydrated culture media does not need to be used all at once; replace the cap and ensure that the container is tightly closed and stored according to labelled instructions.

Dispose of in accordance with local and national authority requirements.

16. References

1. Dang, B. and Graham, D. (2017) *Helicobacter pylori* infection and antibiotic resistance: a WHO high priority? Nat. Rev. Gastroenterol. Hepatol., 14(7), pp.383-384.
2. Fera, M., Carbone, M. and Foca, A. (1993) Activity of cefixime against *Helicobacter pylori*. Int J Antimicrob Agents, 3(2), pp.105-108.
3. Dent, J. and McNulty, C. (1988) Evaluation of a new selective medium for *Campylobacter pylori*. Eur J Clin Microbiol Infect Dis, 7(4), pp.555-558.

Version History*

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*Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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TABLE OF APPLICABLE SYMBOLS

REF o REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place